



Novel Association of ABO Histo-blood Group Antigen with Soluble ICAM-1: Results of a Genome-wide Association Study of 6,578 Women

Citation

Pare, Guillaume, Mark Kellogg, Sunita Badola, Joseph P. Miletich, Greg Gibson, Daniel Ian Chasman, Robert Yuk Lung Zee, Nader Rifai, and Paul M. Ridker. 2008. Novel Association of ABO Histo-blood Group Antigen with Soluble ICAM-1: Results of a Genome-wide Association Study of 6,578 Women. PLoS Genetics 4 (7): e1000118.

Published Version

[doi://10.1371/journal.pgen.1000118](https://doi.org/10.1371/journal.pgen.1000118)

Permanent link

<http://nrs.harvard.edu/urn-3:HUL.InstRepos:5129837>

Terms of Use

This article was downloaded from Harvard University's DASH repository, and is made available under the terms and conditions applicable to Other Posted Material, as set forth at <http://nrs.harvard.edu/urn-3:HUL.InstRepos:dash.current.terms-of-use#LAA>

Share Your Story

The Harvard community has made this article openly available.
Please share how this access benefits you. [Submit a story](#).

[Accessibility](#)

Novel Association of ABO Histo-Blood Group Antigen with Soluble ICAM-1: Results of a Genome-Wide Association Study of 6,578 Women

Guillaume Paré^{1,2*}, Daniel I. Chasman^{1,2}, Mark Kellogg³, Robert Y. L. Zee^{1,2}, Nader Rifai³, Sunita Badola⁴, Joseph P. Miletich⁴, Paul M. Ridker^{1,2}

1 Center for Cardiovascular Disease Prevention, Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts, United States of America, **2** Donald W. Reynolds Center for Cardiovascular Research, Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts, United States of America, **3** Department of Laboratory Medicine, Children's Hospital, Harvard Medical School, Boston, Massachusetts, United States of America, **4** Amgen, Inc., Cambridge, Massachusetts, United States of America

Abstract

While circulating levels of soluble Intercellular Adhesion Molecule 1 (sICAM-1) have been associated with diverse conditions including myocardial infarction, stroke, malaria, and diabetes, comprehensive analysis of the common genetic determinants of sICAM-1 is not available. In a genome-wide association study conducted among 6,578 participants in the Women's Genome Health Study, we find that three SNPs at the ICAM1 (19p13.2) locus (rs1799969, rs5498 and rs281437) are non-redundantly associated with plasma sICAM-1 concentrations at a genome-wide significance level ($P < 5 \times 10^{-8}$), thus extending prior results from linkage and candidate gene studies. We also find that a single SNP (rs507666, $P = 5.1 \times 10^{-29}$) at the ABO (9q34.2) locus is highly correlated with sICAM-1 concentrations. The novel association at the ABO locus provides evidence for a previously unknown regulatory role of histo-blood group antigens in inflammatory adhesion processes.

Citation: Paré G, Chasman DI, Kellogg M, Zee RYL, Rifai N, et al. (2008) Novel Association of ABO Histo-Blood Group Antigen with Soluble ICAM-1: Results of a Genome-Wide Association Study of 6,578 Women. *PLoS Genet* 4(7): e1000118. doi:10.1371/journal.pgen.1000118

Editor: Greg Gibson, The University of Queensland, Australia

Received: March 28, 2008; **Accepted:** June 4, 2008; **Published:** July 4, 2008

Copyright: © 2008 Paré et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Funding: This study was supported by grants from the National Heart Lung and Blood Institute and the National Cancer Institute (Bethesda, MD), the Donald W Reynolds Foundation (Las Vegas, NV), the Doris Duke Charitable Foundation, and the Foundation Leducq (Paris, FR), with collaborative scientific and genotypic support provided by Amgen, Inc. GP is a recipient of a Fonds de la Recherche en Sante du Quebec scholarship. None of these organizations had any influence on data analysis or on the preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

* E-mail: gpare@rics.bwh.harvard.edu

Introduction

ICAM-1 is a member of the immunoglobulin superfamily of adhesion receptors and consists of 5 immunoglobulin-like extracellular domains, a transmembrane domain and a short cytoplasmic domain. ICAM-1, present on endothelial cells, serves as a receptor for the leukocyte integrins LFA-1 (lymphocyte function-associated antigen-1) and Mac-1 (CD11b/CD18), facilitating leukocyte adhesion and migration across the endothelium [1]. A soluble form of ICAM-1 (sICAM-1) is found in plasma and consists of the extracellular domains of ICAM-1. Although the process leading to the formation of sICAM-1 is not entirely clear, sICAM-1 is thought to be shed from the cell membrane via proteolytic cleavage of ICAM-1. Because sICAM-1 binds to LFA-1, it is capable of inhibiting lymphocyte attachment to endothelial cells [2]. Furthermore, sICAM-1 has been shown to bind human rhinoviruses, the etiologic agent of 40–50% of common colds, and to inhibit rhinovirus infection *in vitro* [3]. Likewise, a circulating fragment of sICAM-1 binds to erythrocytes infected with *Plasmodium falciparum*, the etiologic agent of malaria [4] (MIM 611162). Finally, plasma concentration of sICAM-1 has been shown to provide unique predictive value for the risk of myocardial infarction (MIM 608446), ischemic stroke (MIM 601367), peripheral arterial disease (MIM 606787) and noninsulin-dependent diabetes mellitus (MIM 125853) in epidemiological studies [5–7].

Despite relatively high heritability estimates (from 0.34 to 0.59) [8,9] for sICAM-1, few genetic variants are known to influence its concentrations. Two recent linkage studies have shown evidence for genetic association at the ICAM1 (GeneID 3383) locus (19p13.3-p13.2) [8,9] and two candidate SNPs within the extracellular domains of ICAM-1 itself, G241R (rs1799969) and K469E (rs5498), have been correlated with circulating sICAM-1 levels [10,11]. By contrast, a recent genome wide association study (GWAS) from the Framingham investigators involving 1006 participants and 70,987 SNPs revealed no association reaching a genome-wide level of significance, including the ICAM1 locus itself, although this study had no genetic marker within 60 kb of the gene [12]. To more comprehensively explore this issue, we performed a larger GWAS, evaluating 336,108 SNPs in 6,578 apparently healthy women.

Methods

Study Sample and sICAM-1 Measurements

All participants in this study were part of the Women's Genome Health Study (WGHS) [13]. Briefly, participants in the WGHS include American women from the Women's Health Study (WHS) with no prior history of cardiovascular disease, diabetes, cancer, or other major chronic illness who also provided a baseline blood sample at the time of study enrollment. The WHS is a recently

Author Summary

Soluble Intercellular Adhesion Molecule 1 (sICAM-1) is an inflammatory marker that has been associated with several common diseases such as diabetes, heart disease, stroke, and malaria. While it is known that blood concentrations of sICAM-1 are at least partially genetically determined, our current knowledge of which genes mediate this effect is limited. Taking advantage of new technologies allowing us to interrogate genetic variation on a whole genome basis, we found that a variation in the ABO gene is an important determinant of sICAM-1 blood concentrations. Since the ABO gene is responsible for the ABO blood groups, this discovery sheds light on a new role for blood groups and offers novel mechanisms to explain the association between sICAM-1 blood concentrations and various common diseases.

completed 2×2 randomized clinical trial of low-dose aspirin and vitamin E in the primary prevention of cardiovascular disease and cancer. For all WGHS participants, EDTA anticoagulated plasma samples were collected at baseline and stored in vapor phase liquid nitrogen (−170°C). Circulating plasma sICAM-1 concentrations were determined using a commercial ELISA assay (R&D Systems, Minneapolis, Minn.); the assay used is known not to recognize the K56M (rs5491) variant of ICAM-1 [14] and the 22 carriers of this mutation were therefore excluded from further analysis. This study has been approved by the institutional review board of the Brigham and Women's Hospital. Additional clinical characteristics of these subsets are provided in Table S1.

Genotyping

Genotyping was performed in two stages, a first sample being used to discover new associated loci and the second sample being used to validate them by replication. These two samples were genotyped independently of one another in two batches. The first (WGHS-1) and second (WGHS-2) batches included 4,925 and 2,056 self-reported Caucasian WGHS participants, respectively. No related individuals were detected when tested with an identity by state analysis [15].

Samples were genotyped with the Infinium II technology from Illumina. Either the HumanHap300 Duo-Plus chip or the combination of the HumanHap300 Duo and I-Select chips was used. In either case, the custom content was identical and consisted of candidate SNPs chosen without regard to allele frequency to increase coverage of genetic variation with impact on biological function including metabolism, inflammation or cardiovascular diseases. Genotyping at 318,237 HumanHap300 Duo SNPs and 45,571 custom content SNPs was attempted, for a total of 363,808 SNPs. Genetic context for all annotations are derived from human genome build 36.1 and dbSNP build 126.

SNPs with call rates <90% were excluded from further analysis. Likewise, all samples with percentage of missing genotypes higher than 2% were removed. Among retained samples, SNPs were further evaluated for deviation from Hardy-Weinberg equilibrium using an exact method [16] and were excluded when the P-value was lower than 10^{-6} . Samples were further validated by comparison of genotypes at 44 SNPs that had been previously ascertained using alternative technologies. SNPs with minor allele frequency >1% in Caucasians were used for analysis. After quality control, 307,748 HumanHap300 Duo SNPs and 28,360 custom content SNPs were left, for a total of 336,108 SNPs. From the initial 4925 WGHS-1 and 2056 WGHS-2 individuals genotyped,

4582 WGHS-1 individuals and 2014 WGHS-2 individuals were kept for further analysis.

Population Stratification

Because population stratification can result in inflated type I error, a principal component analysis using 1443 ancestry informative SNPs was performed using PLINK [17] in order to confirm self-reported ancestry. Briefly, these SNPs were chosen based on $F_{st} > 0.4$ in HapMap populations (YRB, CEU, CHB+JPT) and inter-SNP distance at least 500 kb in order to minimize linkage disequilibrium. Different ethnic groups were clearly distinguished with the two first components. Out of 4582 WGHS-1 and 2014 WGHS-2 self-identified Caucasians, 12 and 6 were removed from analysis because they did not cluster with other Caucasians, leaving 4570 (WGHS-1) and 2008 (WGHS-2) participants for analysis, respectively. Two more analyses were undertaken to rule out the possibility that residual stratification within Caucasians was responsible for the associations observed. First, association analysis was done with correction by genomic control. This method estimates the average effect of population substructure in the sample (based on median T values) and accordingly corrects the test statistics [18]. Second, a principal component analysis [19] was performed in Caucasians (only) using 124,931 SNPs chosen to have pair-wise linkage disequilibrium lower than $r^2 = 0.4$. The first three components were then used as covariates in the association analysis. As adjustment by these covariates did not change the conclusions, we present analysis among the WGHS-1 and WGHS-2 Caucasian participants without further correction for sub-Caucasian ancestry unless stated otherwise.

Association Analysis

To identify common genetic variants influencing sICAM-1 levels, we first attempted to discover which loci significantly contributed to sICAM-1 concentrations in WGHS-1. Plasma concentrations of sICAM-1 were adjusted for age, smoking, menopause and body mass index using a linear regression model in R to reduce the impact of clinical covariates on sICAM-1 variance. The adjusted sICAM-1 values were then tested for association with SNP genotypes by linear regression in PLINK [17], assuming an additive contribution of each minor allele. A conservative P-value cut-off of 5×10^{-8} was used to correct for the roughly 1,000,000 independent statistical tests thought to correspond to all the common genetic variation of the human genome [20]. Replication of genome-wide significant associations was performed on adjusted sICAM-1 values from the replication sample (WGHS-2), using a Bonferroni correction to account for multiple hypothesis testing.

Model Selection Algorithm

To further define the extent of genetic associations, a forward selection linear multiple regression model was used at the previously identified loci. Briefly, all genotyped SNPs within 100 kb of the most significantly associated SNP at each replicated locus and passing quality control requirements were tested for possible incorporation into a multiple regression model. In stepwise fashion, a SNP was added to the model if its multiple regression P-value was less than 10^{-4} (to account for all the SNPs being considered) and if it had the smallest P-value among all the SNPs not yet included in the model. This analysis was done on WGHS-1 individuals using adjusted sICAM-1 values.

We then proceeded to validate our multiple regression model in WGHS-2 samples. Using only the SNPs previously selected in WGHS-1, we added them in a multiple regression model in the same order as they were chosen in WGHS-1. We considered the model validated if each time a SNP was included in the model, its

regression P-value was lower than 0.01 (to account for multiple testing) and the direction of effect consistent.

Analytical Interference Assay

Plasma from A blood group individuals was mixed 1:1 or 1:2 with a monoclonal anti-A antibody (Ortho-Clinical Diagnostics, Rochester NY), and allowed to incubate 10 minutes or 60 minutes at room temperature, or 60 minutes or 12 hours at 4°C before assaying sICAM-1 levels by the standard technique. To exclude the possibility that the antibody itself interfered with the assay, the same procedure was repeated with plasma from O blood group individuals. Finally, plasma from O group individuals, which is expected to contain both anti-A and anti-B polyclonal antibodies, was mixed with plasma from A group individuals in 1:1 ratio, again with incubation as above and measurement of sICAM-1 levels.

Results/Discussion

As shown in Table 1, 19 SNPs passed our stringent genome-wide significance threshold when tested in WGHS-1 individuals, clustering within two loci in the vicinity of the ICAM1 (19p13.2) and ABO (GeneID 28) (9q34.2) genes (Figure 1). The replication threshold in WGHS-2 was conservatively set at a 2-sided P-value of 0.002, applying a Bonferroni correction to account for 19 tests. Using this cutoff, we were able to replicate 17 of the 19 associated SNPs, including SNPs at both the ICAM1 and ABO loci. Only rs2116941 (19p13.2) and rs7256672 (19p13.2) did not replicate using this standard. Nevertheless, each of these SNPs had a P-value lower than 10^{-9} when tested on the combined sample (i.e. WGHS-1 and WGHS-2 pooled together). Among the replicated SNPs, only rs7258015 (19p13.2) deviated from Hardy-Weinberg equilibrium ($p = 0.00007$), but visual inspection of the raw genotyping signal for this SNP did not reveal any obvious artifact. Major and minor alleles are shown in Table S2.

We then applied our model selection algorithm in WGHS-1 individuals (see Methods) using 54 SNPs at 19p13.2 (ICAM1 locus) and 68 SNPs at 9q34.2 (ABO locus). As can be seen in Table 2, 3 out of 54 SNPs at 19p13.2 were selected by our algorithm and 1 out of 68 SNPs at 9q34.2 was selected. All four SNPs selected in WGHS-1 were validated in WGHS-2. Pairwise linkage disequilibrium between these SNPs was low. For instance, r^2 was lower than 0.35 between ICAM1 SNPs while it was lower than 0.002 between the ABO SNP rs507666 and the ICAM1 SNPs. Among these SNPs, there was no strong evidence for non-additive effects of the minor allele as judged by lack of significance for a likelihood ratio test comparing the additive regression model to an alternative genotype model with an additional degree of freedom. Interestingly, one of the four selected SNPs (rs281437) was non-significant in univariate analysis, illustrating that its inclusion in the model and significant association are conditional on the genotypes at rs5498 and rs281437. No gene-gene interaction was observed between ICAM1 and ABO SNPs. The 3 SNPs at 19p13.2 (ICAM1) collectively explained 6.9% of the total variance in sICAM-1 concentrations (pooling WGHS-1 and WGHS-2 together), whereas the ABO SNP rs507666 explained 1.5%. In comparison, clinical covariates accounted for 18.8% of the variance (Table 3), and together the candidate loci and the clinical variables accounted for 27.3% of total variance. It should be noted that the estimated effect sizes of the ICAM1 and ABO loci are minimums since the genotyped variants might not be the actual functional variants.

The 3 SNPs at the 19p13.2 (ICAM1) locus selected by our algorithm were also used in haplotype analysis using WHAP [21], as implemented in PLINK [17] (Table 4). The estimate of the

proportion of variance attributable to haplotypes, as well as their regression coefficients, is consistent with the linear model of these same SNPs, reinforcing the adequacy of a strictly additive model to explain the association.

The ABO histo-blood group antigen is the most important blood group system in transfusion medicine. Using data from Seattle SNPs (<http://pga.mbt.washington.edu>) as well as from the Blood Group Antigen Mutation Database (www.ncbi.nlm.nih.gov), it can be demonstrated that rs507666 is a perfect surrogate for type A1 histo-blood group antigen. Moreover, using rs687289 as a marker for the O allele, rs8176746 for the B allele and rs8176704 for the A2 allele, complete blood group antigen phenotype can be re-constructed by haplotype analysis (no serotype data is available in WGHS). Imputed haplotypes perfectly fitted the pattern expected from the literature and their association with sICAM-1 is shown in Tables 5 and 6. The A1 allele is associated with the lowest sICAM-1 concentrations while the A2 allele is associated with low concentrations, intermediate between the A1 and O allele. In comparison, the B allele is associated with slightly higher concentrations than the O allele.

Because ABO histo-blood group antigens are known to vary in frequency among Caucasian sub-populations, we sought to investigate the potential effect of population stratification on the observed association even though adjustment of sICAM-1 values for the top ten components of our principal component analysis did not change our conclusions (see Methods). Visual inspection of the clustering pattern from the top two components confirmed a match with previously published work of sub-Caucasian stratification [22] (data not shown). Since these two components were reproducibly shown to correspond to a Northwest-Southeast European gradient [22] and the A1 allele follows such a gradient [23], we hypothesized that they would be tightly linked to A1 allele frequencies. Indeed, the second component showed evidence of association with A1 allelic frequencies ($p = 2.5 \times 10^{-6}$), while the first component was only weakly associated ($p = 0.08$). Nevertheless, neither the first nor second component was very tightly linked to sICAM-1 values ($p = 0.69$ and 0.0006 respectively with corresponding R^2 of 3.8×10^{-5} and 0.0019), implying that stratification has no major effect on the sICAM-1 association. Furthermore, the weak association with the second component could be partially explained by the correlation with A1 alleles, with corrected P-value of 0.004 and R^2 of 0.0013. Adjustment of sICAM-1 values for the first and second components did not substantially change the association between the A1 allele and sICAM-1 (unadjusted $p = 5.1 \times 10^{-29}$ and adjusted $p = 5.5 \times 10^{-28}$), demonstrating that stratification on a Northwest-Southeast European axis is not responsible for the association. We conclude that the data does not support the hypothesis that Northwest-Southeast sub-Caucasian stratification is responsible for the association of ABO variants with sICAM-1 concentrations since the A1 allele varies in frequency according to a Northwest-Southeast European axis while the slight variation in sICAM-1 among this same axis is at least partially dependent on the A1 allele. Indeed, there is no evidence in the literature that mean sICAM-1 concentrations vary at all among Caucasian sub-populations, and this lack of evidence is supported by an overall R^2 of 0.005 (P-value of 0.0007) for the association between sICAM-1 concentrations and the top 10 principal components.

The Secretor phenotype (as defined by rs601338 on chromosome 19q13.33) and the Lewis antigen phenotype (as defined by rs812936 on chromosome 19p13.3) are additional important members of the histo-blood group antigen system. These were therefore tested for association with sICAM-1 levels as well as for interaction with rs507666. No significant effect was observed.

Table 1. Genome-Wide Significant SNPs for sICAM1.

SNP	Locus	Position(kb)	Nearest Gene	Function	MAF ^a	HW ^b	WGHS-1		WGHS-2		Combined		Median sICAM-1 umol/L (n)		
							Beta	P-Value	Beta	P-Value	Beta	P-Value	A1/A1 ^c	A1/A2 ^c	A2/A2 ^c
rs687621	9q34.2	135126.9	ABO	intron	0.34	0.08	-10.88	1.3E-11	-11.21	1.4E-06	-10.95	1.3E-16	343 (2856)	334 (2894)	328 (806)
rs687289	9q34.2	135126.9	ABO	intron	0.34	0.07	-11.00	8.2E-12	-10.81	3.5E-06	-10.91	1.7E-16	343 (2859)	334 (2887)	328 (804)
rs657152	9q34.2	135129.1	ABO	intron	0.37	0.13	-10.17	1.7E-10	-9.93	1.5E-05	-10.06	1.5E-14	343 (2652)	334 (2992)	331 (905)
rs500498	9q34.2	135138.5	ABO	intron	0.45	0.10	9.53	5.0E-10	9.53	2.4E-05	9.52	6.1E-14	332 (2017)	337 (3160)	347 (1358)
rs505922	9q34.2	135139.0	ABO	intron	0.34	0.05	-11.15	4.5E-12	-10.83	3.2E-06	-11.02	9.2E-17	343 (2876)	334 (2871)	328 (798)
rs507666	9q34.2	135139.2	ABO	intron	0.20	0.42	-18.12	1.1E-20	-16.93	5.9E-10	-17.73	5.1E-29	343 (4221)	328 (2055)	314 (268)
rs10409243	19p13.2	10194.0	EDG5	3' UTR ^d	0.41	0.96	-10.93	4.4E-12	-8.22	2.6E-04	-10.11	5.4E-15	344 (2313)	335 (3158)	329 (1083)
rs2116941	19p13.2	10195.4	EDG5	3' UTR ^d	0.20	0.76	-12.05	7.9E-10	-7.47	7.5E-03	-10.67	3.1E-11	341 (4209)	333 (2076)	320 (249)
rs8111930	19p13.2	10229.0	MRPL4	intron	0.12	0.27	18.88	4.4E-15	13.35	4.3E-05	17.10	1.3E-18	335 (5031)	346 (1369)	377 (102)
rs1799969	19p13.2	10255.8	ICAM1	NS ^e	0.12	0.34	-26.11	2.1E-28	-33.18	1.3E-21	-28.19	3.6E-47	343 (5117)	319 (1336)	304 (99)
rs5498	19p13.2	10256.7	ICAM1	NS ^e	0.43	0.15	13.88	6.2E-19	11.78	1.2E-07	13.22	4.8E-25	329 (2117)	338 (3123)	355 (1235)
rs923366	19p13.2	10258.2	ICAM1	3' UTR ^d	0.44	0.71	13.07	5.7E-17	11.05	7.9E-07	12.46	2.5E-22	329 (2088)	338 (3202)	353 (1243)
rs3093030	19p13.2	10258.4	ICAM1	-	0.43	0.59	13.14	3.7E-17	11.41	2.7E-07	12.61	5.9E-23	329 (2109)	338 (3204)	353 (1243)
rs281440	19p13.2	10261.3	ICAM5	-	0.22	0.69	-13.39	7.2E-13	-10.99	4.9E-05	-12.69	1.5E-16	343 (4021)	331 (2223)	322 (314)
rs2075741	19p13.2	10262.1	ICAM5	intron	0.43	0.69	13.27	1.9E-17	11.40	3.2E-07	12.69	3.6E-23	329 (2104)	338 (3208)	353 (1240)
rs2278442	19p13.2	10305.8	ICAM3	intron	0.35	0.65	-9.44	1.0E-08	-7.66	7.7E-04	-8.88	3.1E-11	344 (2727)	334 (3007)	332 (815)
rs2304237	19p13.2	10307.6	ICAM3	NS ^e	0.22	0.49	10.83	1.2E-08	12.23	5.2E-06	11.26	3.9E-13	334 (3919)	342 (2129)	356 (313)
rs7258015	19p13.2	10310.4	ICAM3	NS ^e	0.22	0.00007	10.60	3.2E-08	12.73	8.6E-06	11.19	2.1E-12	334 (3873)	342 (2275)	356 (255)
rs7256672	19p13.2	10440.5	PDE4A	3' UTR ^d	0.36	0.67	-9.06	2.7E-08	-6.36	5.8E-03	-8.24	6.3E-10	343 (2655)	337 (3049)	324 (845)

^aMAF: Minor allele frequency based on the combined samples.^bHW: Deviation from Hardy-Weinberg equilibrium P-value based on the combined samples.^cA1: Major Allele; A2: Minor Allele; Median sICAM-1 values were determined on the combined samples.^d3' UTR: 3' Untranslated Region.^eNS: Non-Synonymous Coding SNP.

doi:10.1371/journal.pgen.1000118.t001

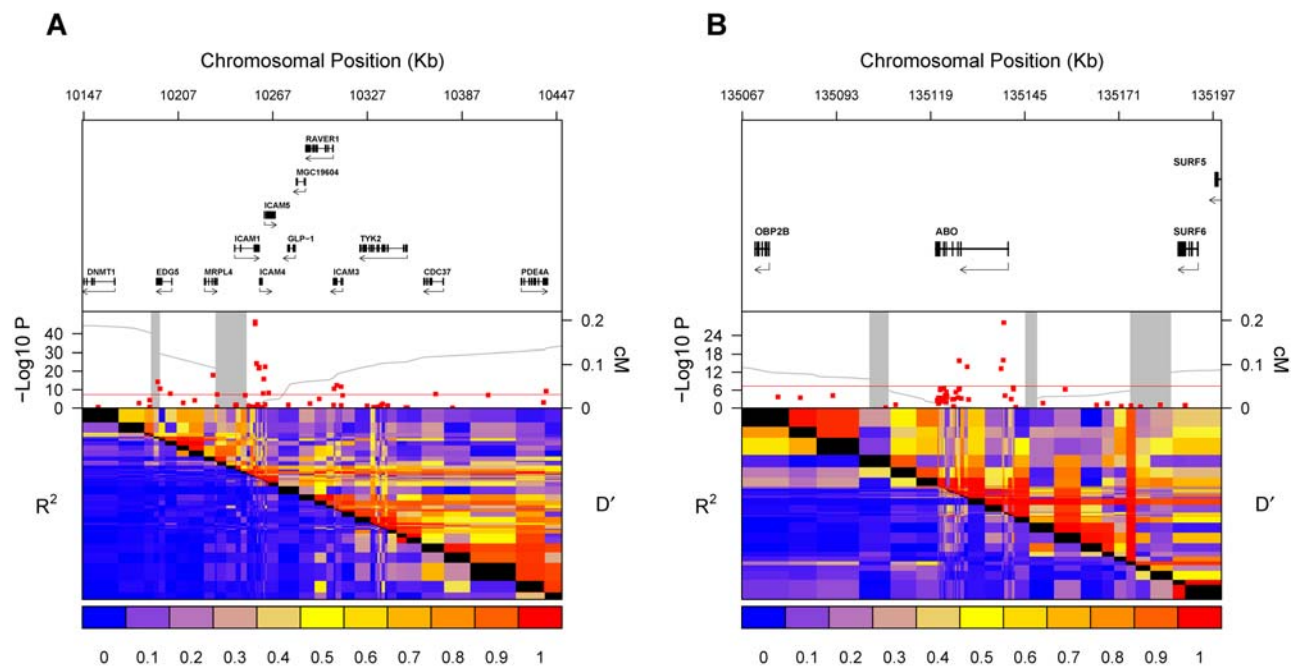


Figure 1. Genetic Context of Genome-Wide Significant Associations. Genomic context for each of two loci with genome-wide association with sICAM-1 levels. The ICAM1 locus (19p13.2) is shown in Figure 1-A and the ABO locus (9q34.2) in Figure 1-B. Upper panel: Genes from RefSeq release 25. Only one isoform is shown when multiple splicing variants are known. Middle Panel: SNPs are shown according to their physical location and P-values (red dots). Also shown is the genetic distance in cM from the lowest P-value SNP (light grey line) along with the position of recombination hotspots (light grey vertical bars). Recombination rates and hotspots are based on HapMap data, as described by McVean et al.[53] and Winckler et al. [54]. Lower panel: Pair wise linkage disequilibrium (D' and R^2) between SNPs based on WGHS data.
doi:10.1371/journal.pgen.1000118.g001

Although the sICAM-1 molecule itself is not known to bear the ABO histo-blood group antigen, this possibility could not be ruled out, especially given its extensive glycosylation [24,25]. We therefore sought to exclude the remote chance that the association between A histo-blood group antigen and lower sICAM-1 values was the consequence of a lower affinity of the antibodies used in the sICAM-1 assay for sICAM-1 carrying the A antigen. In other words, if sICAM-1 does carry ABO histo-blood group antigen, then the allelic composition at the ABO locus could dictate the glycosylation status of the sICAM-1 molecule and possibly interfere with the immunoassay used. While there is no evidence that the two plasma proteins known to contain ABO histo-blood group antigen (von Willebrand factor and alpha 2-macroglobulin) [26] suffer from such analytical interference, immunoassays are

potentially susceptible to differential glycosylation of their target protein [27]. We thus hypothesized that blocking the A antigen sites with either polyclonal or monoclonal antibodies would result in spuriously low sICAM-1 values if sICAM-1 does indeed carry ABO histo-blood group antigen and if the A antigen is located in the vicinity of one of the two antibody binding sites used by the immunoassay. No differential effects of the mixing procedures (see Methods) were observed suggesting that the A blood group antigen was not interfering with measurement of sICAM-1 levels. We therefore conclude that the genetic association of the ABO variant is not due to analytic interference. However, we can not exclude that sICAM-1 bears the ABO histo-blood group antigen.

Finally, we sought to assess the presence of other associations that did not pass our stringent genome-wide P-value cut-off. We

Table 2. Multiple Linear Regression Statistics of SNPs Retained by the Forward Model Selection Algorithm.

Locus	SNP	Nearest Gene	Function	MAF ^a	HW ^b	WGHS-1 ^c		WGHS-2 ^c		Combined ^c	
						Beta	P-Value	Beta	P-Value	Beta	P-Value
19p13.2	rs1799969	ICAM1	NS ^d	0.12	0.34	-41.2	<2.0E-16	-49.3	<2.0E-16	-43.5	<2.0E-16
	rs5498	ICAM1	NS ^d	0.43	0.15	30.4	<2.0E-16	28.3	<2.0E-16	29.7	<2.0E-16
	rs281437	ICAM1	3' UTR ^e	0.30	0.47	11.2	1.3E-08	7.9	5.1E-03	10.1	3.2E-10
9q34.2	rs507666	ABO	intron	0.20	0.42	-16.8	<2.0E-16	-16.8	1.9E-10	-16.7	<2.0E-16

^aMAF: Minor allele frequency based on the combined samples.

^bHW: Deviation from Hardy-Weinberg equilibrium P-value based on the combined samples.

^cAll analyses were performed using adjusted sICAM1 values (see text for details). Beta coefficients and P-values were derived from a multiple linear model that included all 4 SNPs.

^dNS: Non-Synonymous Coding SNP.

^e3' UTR: 3' Untranslated Region.

doi:10.1371/journal.pgen.1000118.t002

Table 3. Partition of sICAM-1 Variance According to Genetic and Clinical Variables.

Category	Variable	Variable R ²	Category R ²
Clinical Covariates	Age	0.012	0.188
	Body Mass Index	0.035	
	Menopause Status	0.006	
	Smoking	0.135	
9q34.2 (ABO) Locus	rs507666	0.015	0.015
19p13.2 (ICAM1) Locus	rs1799969	0.026	0.069
	rs5498	0.039	
	rs281437	0.005	
TOTAL			0.273

doi:10.1371/journal.pgen.1000118.t003

Table 4. Haplotype Analysis of rs1799969, rs5498 and rs281437 (19p13.2; ICAM1 Locus).

Haplotype	Frequency	Beta	P-Value
rs1799969 rs5498 rs281437			
A G G	0.12	−43.91	1.7E-95
G A G	0.27	−30.16	3.0E-78
G A A	0.30	−19.98	5.3E-37
G G G	0.32	Reference	-

Omnibus (3 df) p-value = 1.0E-124. WGHS-1 and WGHS-2 were combined for this analysis.

doi:10.1371/journal.pgen.1000118.t004

therefore repeated the whole-genome association analysis on the combined sample (i.e. WGHS-1 and WGHS-2 pooled together). While no new locus was associated at a genome-wide level, rs9889486 had the lowest p-value (outside of 9q34.2 and 19p13.2; $p = 3.2 \times 10^{-6}$) with a false discovery rate [28] of 0.03. This SNP is intronic to CCDC46 (GeneID 201134) (17q24.1), a gene whose function is not well characterized. Among other low p-value SNPs, we note rs1049728 ($p = 1.3 \times 10^{-5}$) with a false discovery rate of 0.08 and the 51st most strongly associated SNP overall. This SNP is located in the 3' untranslated region of RELA (GeneID 5970) (11q13.1), which is part of the NFκB signaling complex, arguably the most important known regulator of ICAM1 expression [29].

The non-synonymous coding ICAM1 SNPs rs1799969 (G241R) and rs5498 (K469E) were previously described as being associated

Table 6. Mean (SD) Level of sICAM-1 (μmol/L) According to Predicted ABO Alleles.

		First Allele			
		A1	A2	O	B
Second Allele	A1	326 (71) N = 268	332 (71) N = 185	338 (77) N = 1656	352 (89) N = 182
	A2	-	346 (55) N = 33	352 (80) N = 537	366 (91) N = 56
	O	-	-	358 (81) N = 2859	356 (80) N = 592
	B	-	-	-	342 (79) N = 51

WGHS-1 and WGHS-2 were combined for this analysis. Mean (SD) values were derived from unadjusted sICAM-1 concentrations.

doi:10.1371/journal.pgen.1000118.t006

with sICAM-1 levels [10,11] whereas the association involving rs281437 is unreported. The later SNP is in the 3' untranslated region of ICAM-1. Of interest, the minor allele of rs1799969 (arginine) is correlated with lower sICAM-1 and has been associated with lower risk of type I diabetes [30], while the minor allele of rs5498 (glutamic acid) is correlated with higher sICAM-1 levels and has been associated with lower risk of asthma [11] (MIM 600807), inflammatory bowel disease [31] (MIM 266600) and type I diabetes [32] (MIM 222100). Furthermore, it has been demonstrated *in vitro* that this SNP affects ICAM-1 mRNA splicing pattern and apoptosis in human peripheral blood mononuclear cells [33]. It is also noteworthy that sICAM-1 has been shown to inhibit insulinitis and onset of autoimmune diabetes in a mouse model of type I diabetes [34] whereas ICAM1 itself was proven to be crucial to the priming of T cells against beta cells [35].

The most striking result of this report is the association between sICAM-1 levels and rs507666, a SNP intronic to the ABO gene. The ABO gene encodes glycosyltransferase enzymes which transfer specific sugar residues to a precursor substance, the H antigen. There are three major alleles at the ABO locus: A, B and O. Variation at the ABO locus is remarkable in that these alleles encode enzymes with different specificities as well as activities. The A allele encodes the enzyme $\alpha 1 \rightarrow 3$ N-acetylgalactosaminyl-transferase which forms the A antigen from the H antigen. The A allele (as well as the B and O alleles) is itself heterogeneous and comprises several subgroups, of which A1 and A2 are the most important. As compared to A1, the A2 allele has 30–50 fold less A transferase activity [36]. The B allele encodes the enzyme $\alpha 1 \rightarrow 3$ galactosyltransferase which forms the B antigen from the H antigen. The O allele does not produce an active enzyme [37]. Consistent

Table 5. Association of sICAM-1 Concentrations (μmol/L) with Histo-Blood Group Antigen Alleles (9q34.2; ABO Locus).

Allele	Genotype				Frequency	Beta
	rs8176746	rs8176704	rs687289	rs507666		
A1	C	G	A	A	0.20	−14.3
A2	C	A	A	G	0.07	−2.2
B	A	G	A	G	0.08	4.7
O	C	G	G	G	0.66	3.9

Omnibus (3 df) p-value = 2.0E-28. WGHS-1 and WGHS-2 were combined for this analysis.

doi:10.1371/journal.pgen.1000118.t005

with the A antigen being associated with lower sICAM-1 concentrations and with the A1 allele having 30–50 fold more A transferase activity than the A2 allele, the A1 allele is associated with the lowest sICAM-1 concentrations while the A2 allele is associated with low concentrations as well, but still higher than the A1 allele (Table 5). Although we excluded the possibility of an analytical interference to explain the association, the exact mechanism linking histo-blood group antigen to sICAM-1 concentrations remains elusive. Among the different hypotheses, it remains possible that sICAM-1 bears the A antigen, a modification that might increase its clearance by increasing its affinity for its receptor(s) and/or decrease its secretion, perhaps by decreasing its affinity for the protease(s) producing sICAM-1 from membrane-bound ICAM1. Alternatively, lower sICAM-1 concentrations might be the result of the presence of the A antigen on its receptor(s) and/or protease(s).

ABO histo-blood group phenotype has been linked to a plethora of diseases, including infectious diseases, cancers and vascular diseases [38]. Particularly interesting is the association of non-O histo-blood groups — and group A in particular [39,40] — with a higher risk of myocardial infarction, peripheral vascular disease, strokes and venous thromboembolism [41] (MIM 188050). While this phenomenon is partially explained by higher concentrations of the coagulation factors vonWillebrand and VIII (presumably because of decreased clearance) [42], the exact mechanism is not entirely understood. Underlining the complex nature of the biological processes involved, the A1 group (rs507666) is associated with lower levels of sICAM-1, a (positive) predictor of vascular diseases in epidemiological studies [5,6,43–46]. Among potential explanations as to this apparent disparity, it is possible that decreased sICAM-1 leads to increased adhesion of leukocytes on endothelial surface and therefore increased vascular inflammation, an important component of atherosclerosis [47]. Moreover, because group A individuals have been shown to have higher blood cholesterol [48] and coagulability [42], the decrease in sICAM-1 seen in these individuals could be offset by the increased susceptibility to vascular diseases conferred by these risk factors, even if sICAM-1 mechanistically causes these diseases. Alternatively, sICAM-1 might merely be a marker of increased inflammation and coagulation [49], both risk factors for vascular diseases. Also of special interest, group A antigen carriers have been recognized as having a higher risk of suffering from severe malaria when infected by *Plasmodium falciparum* [50]. *Plasmodium* infected erythrocytes express a receptor (PfEMP-1) that binds specifically to cell-surface group A and B antigen as well as ICAM-1 [51], a major step in the sequestration of infected erythrocytes leading to the clinical complications of severe and cerebral

malaria. The lower concentrations of sICAM-1 found in A1 group carriers could therefore be hypothesized to contribute to this higher risk either directly, if sICAM-1 can inhibit the sequestration process, or indirectly, if sICAM-1 levels reflect differences in the processing of the ICAM1 receptor itself.

Several limitations warrant discussion. First, this study was conducted in Caucasian women. It is therefore difficult to generalize our results to other ethnicities or to men. Second, effect estimates derived from this study might be higher than in other populations as these are initial findings and because of the winner's curse [52]. Third, although we were able to rule out a technical artifact as the cause of our results, no mechanistic link is identified to explain the association between ABO histo-blood groups and sICAM-1. In particular, one pending question is whether or not ICAM-1 bears any ABO antigen at all.

In this report, we demonstrate that sICAM-1 concentrations are associated with genetic variation at the ABO and ICAM1 loci in women. To our knowledge, this represents the first published genetic evidence that ABO may have a regulatory role on an inflammatory mediator, a finding with potential implication on a diverse array of immune-mediated disorders. Especially interesting is the fact that both ABO and ICAM1 have been previously related to vascular disease and malaria, two major causes of mortality and morbidity worldwide. The current study indicates a genetic link between histo-blood group antigen and inflammatory adhesion processes, providing the basis for physiological studies of this interaction.

Supporting Information

Table S1 Clinical Characteristics of the Samples Used.

Found at: doi:10.1371/journal.pgen.1000118.s001 (0.04 MB DOC)

Table S2 Major and Minor Alleles.

Found at: doi:10.1371/journal.pgen.1000118.s002 (0.04 MB DOC)

Acknowledgments

We thank the study research team and the women who have participated in the study. We also thank Lynda Rose for her review of the manuscript and helpful comments.

Author Contributions

Conceived and designed the experiments: GP DC MK RZ NR JM PR. Performed the experiments: MK RZ NR SB. Analyzed the data: GP DC. Wrote the paper: GP DC.

References

- van de Stolpe A, van der Saag PT (1996) Inter cellular adhesion molecule-1. *J Mol Med* 74: 13–33.
- Rieckmann P, Michel U, Albrecht M, Bruck W, Wockel L, et al. (1995) Soluble forms of inter cellular adhesion molecule-1 (ICAM-1) block lymphocyte attachment to cerebral endothelial cells. *J Neuroimmunol* 60: 9–15.
- Marlin SD, Staunton DE, Springer TA, Stratowa C, Sommergruber W, et al. (1990) A soluble form of inter cellular adhesion molecule-1 inhibits rhinovirus infection. *Nature* 344: 70–72.
- Ockenhouse CF, Betageri R, Springer TA, Staunton DE (1992) *Plasmodium falciparum*-infected erythrocytes bind ICAM-1 at a site distinct from LFA-1, Mac-1, and human rhinovirus. *Cell* 68: 63–69.
- Ridker PM, Hennekens CH, Roitman-Johnson B, Stampfer MJ, Allen J (1998) Plasma concentration of soluble inter cellular adhesion molecule 1 and risks of future myocardial infarction in apparently healthy men. *Lancet* 351: 88–92.
- Pradhan AD, Rifai N, Ridker PM (2002) Soluble inter cellular adhesion molecule-1, soluble vascular adhesion molecule-1, and the development of symptomatic peripheral arterial disease in men. *Circulation* 106: 820–825.
- Song Y, Manson JE, Tinker L, Rifai N, Cook NR, et al. (2007) Circulating levels of endothelial adhesion molecules and risk of diabetes in an ethnically diverse cohort of women. *Diabetes* 56: 1898–1904.
- Bielinski SJ, Pankow JS, Foster CL, Miller MB, Hopkins PN, et al. (2007) Circulating soluble ICAM-1 levels shows linkage to ICAM gene cluster region on chromosome 19: The NHLBI Family Heart Study follow-up examination. *Atherosclerosis*.
- Kent JW Jr, Mahaney MC, Comuzzie AG, Goring HH, Almay L, et al. (2007) Quantitative trait locus on Chromosome 19 for circulating levels of inter cellular adhesion molecule-1 in Mexican Americans. *Atherosclerosis* 195: 367–373.
- Ponthieux A, Lambert D, Herbeth B, Drosch S, Pfister M, et al. (2003) Association between Gly241Arg ICAM-1 gene polymorphism and serum sICAM-1 concentration in the Stanislas cohort. *Eur J Hum Genet* 11: 679–686.
- Puthothu B, Krueger M, Bernhardt M, Heinzmann A (2006) ICAM1 amino-acid variant K469E is associated with paediatric bronchial asthma and elevated sICAM1 levels. *Genes Immun* 7: 322–326.
- Benjamin EJ, Dupuis J, Larson MG, Lunetta KL, Booth SL, et al. (2007) Genome-wide association with select biomarker traits in the Framingham Heart Study. *BMC Med Genet* 8 Suppl 1: S11.
- Ridker PM, Chasman DI, Zee RY, Parker A, Rose L, et al. (2008) Rationale, Design, and Methodology of the Women's Genome Health Study: A Genome-Wide Association Study of More Than 25 000 Initially Healthy American Women. *Clin Chem* 54: 249–255.

14. Register TC, Burdon KP, Lenchik L, Bowden DW, Hawkins GA, et al. (2004) Variability of serum soluble intercellular adhesion molecule-1 measurements attributable to a common polymorphism. *Clin Chem* 50: 2185–2187.
15. Serre D, Montpetit A, Pare G, Engert JC, Yusuf S, et al. (2008) Correction of population stratification in large multi-ethnic association studies. *PLoS ONE* 3: e1382.
16. Wigginton JE, Cutler DJ, Abecasis GR (2005) A note on exact tests of Hardy-Weinberg equilibrium. *Am J Hum Genet* 76: 887–893.
17. Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, et al. (2007) PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am J Hum Genet* 81: 559–575.
18. Devlin B, Roeder K (1999) Genomic control for association studies. *Biometrics* 55: 997–1004.
19. Price AL, Patterson NJ, Plenge RM, Weinblatt ME, Shadick NA, et al. (2006) Principal components analysis corrects for stratification in genome-wide association studies. *Nat Genet* 38: 904–909.
20. Frazer KA, Ballinger DG, Cox DR, Hinds DA, Stuve LL, et al. (2007) A second generation human haplotype map of over 3.1 million SNPs. *Nature* 449: 851–861.
21. Purcell S, Daly MJ, Sham PC (2007) WHAP: haplotype-based association analysis. *Bioinformatics* 23: 255–256.
22. Price AL, Butler J, Patterson N, Capelli C, Pascali VL, et al. (2008) Discerning the ancestry of European Americans in genetic association studies. *PLoS Genet* 4: e236.
23. Cavalli-Sforza LL, Menozzi P, Piazza A (1994) *The History and Geography of Human Genes*. Princeton: Princeton University Press. 541 p.
24. Diamond MS, Staunton DE, Marlin SD, Springer TA (1991) Binding of the integrin Mac-1 (CD11b/CD18) to the third immunoglobulin-like domain of ICAM-1 (CD54) and its regulation by glycosylation. *Cell* 65: 961–971.
25. Otto VI, Schurpf T, Folkers G, Cummings RD (2004) Sialylated complex-type N-glycans enhance the signaling activity of soluble intercellular adhesion molecule-1 in mouse astrocytes. *J Biol Chem* 279: 35201–35209.
26. Matsui T, Fujimura Y, Nishida S, Titani K (1993) Human plasma alpha 2-macroglobulin and von Willebrand factor possess covalently linked ABO(H) blood group antigens in subjects with corresponding ABO phenotype. *Blood* 82: 663–668.
27. Helander A, Eriksson G, Stibler H, Jeppsson JO (2001) Interference of transferrin isoform types with carbohydrate-deficient transferrin quantification in the identification of alcohol abuse. *Clin Chem* 47: 1225–1233.
28. Benjamini Y, Hochberg Y (1995) Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing. *Journal of the Royal Statistical Society Series B (Methodological)* 57: 289–300.
29. Ledebur HC, Parks TP (1995) Transcriptional regulation of the intercellular adhesion molecule-1 gene by inflammatory cytokines in human endothelial cells. Essential roles of a variant NF-kappa B site and p65 homodimers. *J Biol Chem* 270: 933–943.
30. Nejentsev S, Guja C, McCormack R, Cooper J, Howson JM, et al. (2003) Association of intercellular adhesion molecule-1 gene with type 1 diabetes. *Lancet* 362: 1723–1724.
31. Matsuzawa J, Sugimura K, Matsuda Y, Takazoe M, Ishizuka K, et al. (2003) Association between K469E allele of intercellular adhesion molecule 1 gene and inflammatory bowel disease in a Japanese population. *Gut* 52: 75–78.
32. Ma J, Mollsten A, Prazny M, Falhammar H, Brismar K, et al. (2006) Genetic influences of the intercellular adhesion molecule 1 (ICAM-1) gene polymorphisms in development of Type 1 diabetes and diabetic nephropathy. *Diabet Med* 23: 1093–1099.
33. Iwao M, Morisaki H, Morisaki T (2004) Single-nucleotide polymorphism g.1548G > A (E469K) in human ICAM-1 gene affects mRNA splicing pattern and TPA-induced apoptosis. *Biochem Biophys Res Commun* 317: 729–735.
34. Martin S, Heidenthal E, Schulte B, Rothe H, Kolb H (1998) Soluble forms of intercellular adhesion molecule-1 inhibit insulinitis and onset of autoimmune diabetes. *Diabetologia* 41: 1298–1303.
35. Camacho SA, Heath WR, Carbone FR, Sarvetnick N, LeBon A, et al. (2001) A key role for ICAM-1 in generating effector cells mediating inflammatory responses. *Nat Immunol* 2: 523–529.
36. Yamamoto F, McNeill PD, Hakomori S (1992) Human histo-blood group A2 transferase coded by A2 allele, one of the A subtypes, is characterized by a single base deletion in the coding sequence, which results in an additional domain at the carboxyl terminal. *Biochem Biophys Res Commun* 187: 366–374.
37. Yip SP (2002) Sequence variation at the human ABO locus. *Ann Hum Genet* 66: 1–27.
38. Greenwell P (1997) Blood group antigens: molecules seeking a function? *Glycoconj J* 14: 159–173.
39. Platt D, Muhlberg W, Kiehl L, Schmitt-Ruth R (1985) ABO blood group system, age, sex, risk factors and cardiac infarction. *Arch Gerontol Geriatr* 4: 241–249.
40. Medalie JH, Levene C, Papier C, Goldbourt U, Dreyfuss F, et al. (1971) Blood groups, myocardial infarction and angina pectoris among 10,000 adult males. *N Engl J Med* 285: 1348–1353.
41. Wu O, Bayoumi N, Vickers MA, Clark P (2008) ABO(H) blood groups and vascular disease: a systematic review and meta-analysis. *J Thromb Haemost* 6: 62–69.
42. Jenkins PV, O'Donnell JS (2006) ABO blood group determines plasma von Willebrand factor levels: a biologic function after all? *Transfusion* 46: 1836–1844.
43. de Lemos JA, Hennekens CH, Ridker PM (2000) Plasma concentration of soluble vascular cell adhesion molecule-1 and subsequent cardiovascular risk. *J Am Coll Cardiol* 36: 423–426.
44. O'Malley T, Ludlam CA, Riemersma RA, Fox KA (2001) Early increase in levels of soluble inter-cellular adhesion molecule-1 (sICAM-1); potential risk factor for the acute coronary syndromes. *Eur Heart J* 22: 1226–1234.
45. Tzoulaki I, Murray GD, Lee AJ, Rumley A, Lowe GD, et al. (2005) C-reactive protein, interleukin-6, and soluble adhesion molecules as predictors of progressive peripheral atherosclerosis in the general population: Edinburgh Artery Study. *Circulation* 112: 976–983.
46. Albert MA, Glynn RJ, Buring JE, Ridker PM (2007) Differential effect of soluble intercellular adhesion molecule-1 on the progression of atherosclerosis as compared to arterial thrombosis: A prospective analysis of the Women's Health Study. *Atherosclerosis*.
47. Libby P, Ridker PM, Maseri A (2002) Inflammation and atherosclerosis. *Circulation* 105: 1135–1143.
48. Sing CF, Orr JD (1976) Analysis of genetic and environmental sources of variation in serum cholesterol in Tecumseh, Michigan. III. Identification of genetic effects using 12 polymorphic genetic blood marker systems. *Am J Hum Genet* 28: 453–464.
49. Clark P, Jordan F, Pearson C, Walker ID, Sattar N, et al. (2003) Intercellular adhesion molecule-1 (ICAM-1) expression is upregulated by thrombin in human monocytes and THP-1 cells in vitro and in pregnant subjects in vivo. *Thromb Haemost* 89: 1043–1051.
50. Cserti CM, Dzik WH (2007) The ABO blood group system and Plasmodium falciparum malaria. *Blood* 110: 2250–2258.
51. Smith JD, Craig AG, Kriek N, Hudson-Taylor D, Kyes S, et al. (2000) Identification of a Plasmodium falciparum intercellular adhesion molecule-1 binding domain: a parasite adhesion trait implicated in cerebral malaria. *Proc Natl Acad Sci U S A* 97: 1766–1771.
52. Zollner S, Pritchard JK (2007) Overcoming the winner's curse: estimating penetrance parameters from case-control data. *Am J Hum Genet* 80: 605–615.
53. McVean GA, Myers SR, Hunt S, Deloukas P, Bentley DR, et al. (2004) The fine-scale structure of recombination rate variation in the human genome. *Science* 304: 581–584.
54. Winckler W, Myers SR, Richter DJ, Onofrio RC, McDonald GJ, et al. (2005) Comparison of fine-scale recombination rates in humans and chimpanzees. *Science* 308: 107–111.